MORPHOLOGICAL ANALYSIS OF RETINAL GLIA

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INTRODUCTION: In addition to neuronal cells, vertebrate retinas also contain non-neuronal cells, referred to as glia. The three types of glial cells found in the retina are the Müller glia, retinal astrocytes, and microglia; together these cells aid in function, support, and maintenance of the retina. Glial cells become reactive upon activation induced by disease or injury to the retina. Activation of the cells is accompanied by morphological, molecular, and functional changes; microglial cells increase production of cytokines and convert to macrophage-like behavior to rid the nervous system of debris following disease, while astrocytes de-differentiate, migrate, proliferate and can form permanent barriers to nervous system regeneration, called glial scars. The aim of this project was characterization of the activation states of these various glial cells based on morphological parameters following treatment of cells with vehicle, known activators, and a suspected activator, the growth factor bone morphogenetic protein 7 (BMP7). Morphological changes, such as cell area, branch points, and branch length, which are indicators of a change in the function of the cells brought about by activation, were measured.

METHODS: Cell cultures of mouse retinal astrocytes were treated with vehicle or sodium peroxynitrite (a strong oxidizing agent) while retinal microglia cells *in vitro* were treated with vehicle, lipopolysaccharide (LPS), or BMP7. Immunocytochemistry (ICC) was performed on the treated samples with cell-specific markers. The astrocytes were labeled with glial fibrillary acidic protein (GFAP) and the microglia labeled with ionized calcium-binding adapter molecule 1 (IBA-1) and submitted to immunofluorescence for imaging. Subsequent determination of morphological changes was done through the software Adobe Photoshop CS6 for preprocessing and then FIJI (ImageJ 2.0) for morphological analysis through the ‘Analyze’ feature and ‘Skeleton’ plugin.

RESULTS: ICC analysis demonstrated morphological changes were induced in comparison to vehicle treated controls. The astrocytes treated with sodium peroxynitrite for 32hrs exhibited an increase of 2x in cell area and number of branch points and a decrease of 2x in branch length with increasing branch complexity. The microglia exhibited an increase of 1.2x in cell area, 1.4x in number of branch points and a decrease of 1.2x in branch length at 3hrs and an increase of 1.1x in cell area, 1.6x in number of branch points, and a decrease of 1.3x in branch length at 24hrs with LPS and a 1.5x increase of average cell area and number of branch points and minimal difference in branch length at 24hrs with BMP7.

CONCLUSION: Activation of both astrocytes and microglia by known activators and BMP7 brought about increases in cell area, number of branch points and a decrease in branch length. This evidence is consistent with BMP7 triggering activation in both microglia and retinal astrocytes.